Claims

1. Isolated $CD4^+CD25^-$ T cells which are able to exert contact-independent regulatory functions.

2. The T cells according to claim 1, which

(i) are human $CD4^+CD25^-$ T cells, and/or

(ii) are isolatable from peripheral blood.

3. The T cells according to claim 2, which are isolatable from peripheral blood by suitable monoclonal antibodies and using magnetic separation or immunoadsorption methods.

4. Isolated Tr1-like regulatory T cells which are obtainable by anergizing the $CD4^+CD25^-$ T cells according to claim 1, and exert contact-independent regulatory functions.

5. The T cells of claim 4, which

(i) are human Tr1-like regulatory T cells, and/or

(ii) are capable of IL-10 production.

6. A method for expanding $CD4^+CD25^-$ T cells as defined in claim 1 or Tr1-like regulatory T cells as defined in claim 3, which method comprises stimulating the T cells with a T cell stimulating agent or with antigen-presenting cells *ex vivo* and *in vivo*.

7. The method of claim 6, wherein

(i) the T cell stimulating agent is a composition comprising

(a) anti-CD3 and/or anti-CD28 ligands/monoclonal antibodies,

(b) a ligand/antibody to T cell receptors on the surface of the $CD4^+CD25^+$ T cells or the Tr1-like regulatory T cells, or to T cell receptor components; or

(c) MHC-peptide complexes binding to the T cell receptors expressed on the surface of regulatory T cells; or

(d) a phorbolester and a calcium ionophor, and/or

(ii) the antigen-presenting cells are selected from autologous, non-autologous and artificial antigen-presenting cells.

8. The method according to claim 7, wherein the anti-CD3 and/or anti-CD28 ligands/monoclonal antibodies are superagonistic antibodies, or the antigen-presenting cells are dendritic cells, or the T cells stimulating agent and antigen-presenting cells are used together with IL-2 and/or IL-5, IL-7 and/or IL-9, IFN-α and/or IL-10.

9. A method for producing the Tr1-like regulatory T cells of claim 4, which method comprises anergizing $CD4^+CD25^-$ T cells by contacting the $CD4^+CD25^-$ T cells with an anergic state inducing agent *ex vivo* and *in vivo*.

10. The method of claim 9, wherein

(i) the anergic state inducing agent comprises $CD4^+CD25^+$ T cells; or

(ii) the anergic state inducing agent is a substance or a mixture of substances mimicking the role of $CD4^+CD25^+$ T cells.

11. The method of claim 10, wherein the anergic state inducing agent comprises $CD4^+CD25^+$ T cells and said method comprises co-culturing the $CD4^+CD25^-$ T cells with $CD4^+CD25^+$ T cells.

12. Expanded $CD4^+CD25^-$ T cells and expanded Tr1-like regulatory T cells obtainable by the method according to claim 6.

13. Tr1-like regulatory T cells obtainable by the method according to claim 9.

14. A pharmaceutical composition comprising human $CD4^+CD25^-$ T cells or Tr1-like regulatory T cells according to any one of claims 1, 4, 12 or 13.

15. Method of using $CD4^+CD25^-$ T cells or Tr1-like regulatory T cells according to any one of claims 1, 4, 12 or 13

(f) in assays that will allow an indentification of other regulatory factors;

(g) for identifying molecules expressed by the $CD4^+CD25^-$ T cells or by the Tr1-like regulatory T cells;

(h) for identifying precursor cells or progeny of regulatory $CD4^+CD25^-$ T cells or of Tr1-like regulatory T cells;

(i) for adoptive transfer therapy, for treating diseases with enhanced immunity, or for preventing/treating transplantation reactions.

16. Method according to claim 15, which is

(a) for identifying novel molecules on the $CD4^+CD25^-$ T cells or the Tr1-like regulatory T cells; or

(b) for treating autoimmune diseases; or

(c) for preventing/treating graft versus host disease or graft rejections.

17. Method of using an anergic state inducing agent for inducing Tr1-like regulatory T cells *in vivo*.

18. Method according to claim 17, which is carried out for treating autoimmune diseases in a patient.

19. A method for adoptive transfer therapy which comprises injecting/infusing back into patients enriched/expanded autologous or non-autologous Tr1-like regulatory T cells according to any one of claims 4, 12 or 13.

20. A method for preparing $CD4^+CD25^-$ T cells and Tr1-like regulatory T cells with a particular desired antigen-specific T cell receptor which comprises
(i) activating/stimulating/expanding the $CD4^+CD25^-$ T cells according to claim 1 or the Tr1-like regulatory T cells according to claim 4 with antigen presenting cells, presenting said antigen *in vitro* or *in vivo*, or
(ii) utilizing a ligand/antibody to a particular T cell receptor expressed on (subsets of) $CD4^+CD25^-$ regulatory T cells or Tr1-like regulatory T cells, or a MHC-peptide complex binding to a particular T cell receptor on (subsets of) $CD4^+CD25^-$ T cells or Tr1-like regulatory T cells, and

optionally, in case of $CD4^+CD25^-$ T cells, anergizing said $CD4^+CD25^-$ T cells by contacting them with an anergic state inducing agent.

21. The method of claim 20, wherein the antigen presenting cells are immature or mature dendritic cells (DC).

22. The method of claim 20, wherein the antigen-presenting cells are pulsed/loaded/fed with tissue or any defined or undefined antigens, wherein
(i) the defined antigens are autoantigens delivered as peptide, protein, immune complex, RNA or hybrids between cells of interest and dendritic cells/antigen presenting cells; or
(ii) the undefined antigens are tissue or cell-derived antigens or pathogen-derived antigens.

23. The method of claim 22, wherein the defined antigens are bispecific antibodies bound to antigen and Fc receptors, or the undefined antigens are antigens which are in the form of viable necrotic or apoptotic cells, with or without being complexed to antibodies as defined in (i) above, or tissue derived RNA or hybrids between cells of interest and dendritic cells/antigen presenting cells, other forms of delivery of undefined antigens into dendritic cells or other antigen presenting cells.

24. $CD4^+CD25^-$ T cells and Tr1-like regulatory T cells having a particular desired antigen-specific T cell receptor and obtainable by

(iii) the method of claim 20, or by transfection of a T cell receptor of desired antigen specificity into *ex vivo* isolated or expanded T cells; or

(iv) the method of claim 20, and which have been brought into an anergic state.

25. Pharmaceutical composition comprising the T cells of claim 16.

26. Method of using agents specifically binding to defined entities on the Tr1-like regulatory T cells for removal or functional impairment of Tr1-like regulatory T cells *in vivo* in order to enhance immune responses.

27. The method of claim 26, wherein the agents are selected from monoclonal antibodies or MHC-peptide complexes or other ligands binding to T cell receptors on (subsets of) the Tr1-like regulatory T cells, or the method is performed to dampen regulation by and Tr1-like regulatory T cells *in vivo.*

28. The method of claim 27, which is performed to enhance tumor immunity.